

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1-15. (cancelled)

16. (currently amended) A recombinant nucleic acid molecule encoding a modified type 14 pneumolysin polypeptide comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity wherein at least one amino acid in the region comprising amino acid residues 1 to 257 is substituted and wherein at least one of said amino acid substitutions results in attenuation of the hemolytic activity of the modified pneumolysin polypeptide.

17. (currently amended) The recombinant nucleic acid molecule according to claim 16 comprising the ~~following~~ pneumolysin nucleic acid sequence of SEQ ID NO: 1, [[:]

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ATGCGAAATA AAGCAGTAAA TCACTTTATA CTAGCTATGA 40
ATTACGATAA AAACAAACTC TTGACCCATC AGGCAGAAAG 80
TATTGAAAAT CGTTTCATCA AAGAGCGTAA TCAGCTACCC 120
GATGAGTTTG TTGTTATCGA AAGAAAGAAG CCGAGCTTGT 160
CGACAAATAC AAGTGATATT TCTGTAACAG CTACCAACGA 200
CAGTCGCCCTC TATCCTCGAG CACTTCTCGT AGTCGATGAG 240
ACCTTGTTAG AGAATAATCC CACTCTTCTT CCGCTCGATC 280
GTGCTCCGAT CACTTATAGT ATTGATTTCG CTGGTTTGCC 320
AAGTAGCCAT AGCTTTCTCC AAGTGCAAGA TCCCAGCAAT 360
TCAAGTGTTT CCGCAGCCGT AAACGATTTG TTGGCTAAGT 400
GGCATCAAGA TTATGCTCAG GTCAATAATG TCCCAGCTAG 440
AATGCAGTAT GAAAAAATCA CCGCTCACAG CATCGAACAA 480
CTCAAGGTCA AGTTTGGTTC TGACTTTGAA AAGACAGCGA 520
ATTCTCTTGA TATTGATTTT AACTCTCTCC ATTCAGGCCA 560
AAAGCAGATT CAGATTGTTA ATTTTAAGCA GATTTATTAT 600
ACACTCAGCG TAGACGCTGT TAAAAATCCA CGAGATGTGT 640
TTCAAGATAC TGTAACGCTA GAGGATTTAA AACACAGAGG 680
AATTTCTGCA GAGCGTCCTT TCGTCTATAT TTCGAGTGTT 720
GCTTATGGGC GCCAAGTCTA TCTCAAGTTG GAAACCACCA 760
GTAAGACTCA TGAAGTAGAG CCTGCTTTTG AAGCTTTTCAT 800
AAAAGCAGTC AAGCTAGCTC CTCACACACA GTCGAAGCAG 840
ATTTTGGACA ATACAGAAGT GAAGCGGTTT ATTTTACGGG 880
GGGACCCAAG TTCGGGTGCC CGAGTTGTAA CAGGCAAGCT 920

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GGATATGCTA GAGGACTTGA TTCAAGAAGG CAGTCGCTTT          960
ACAGCAGATC ATCCAGGCTT GCCGATTTC TATACAACCT          1000
CTTTTTTTACG TGACAATGTA GTTGGCACCCT TTCAAAATAG          1040
TACAGACTAT GTTCAGACTA AGCTTACAGC TTACAGAAAC          1080
GCAGATTTAC TGCTGCATCA TAGTGCTGCC TATGTTGCCC          1120
AATATTATAT TACTTGCAAT GAATTATCCT ATGATCATCA          1160
AGCTAAGCAA CTCTTGACTC CTAAGGCTTG CGACAGAAAT          1200
GGGCAGGATT TAACGGCTCA CTTTACCACT AGTATTCCTT          1240
TAAAAGGGAA TGTTCTTAAT CTCTCTGTCA AAATTAGACA          1280
GTGTACCGGG CTTGCTTGGG AATGGTGGCG TACGGTTTAT          1320
GAAAAAACCG ATTTGCCACT AGTGGCTAAG CGGACGATTT          1360
CTATTTGGCG AACAACTCTC TATCCGCAGC TAGAAGATAA          1400
GCTAGAAAAT GAC          (SEQ ID NO:1)          1413

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and wherein said nucleic acid sequence comprises one or more of the nucleotide substitutions selected from the group consisting of:

A-50→G, G-54→T, T-181→C, A-196→T and T-302→C;

A-122→G, A-514→G, T-583→A and A-764→G;

A-187→T, T-380→A, A-382→C and T-443→A;

T-98→C, T-137→C, T-248→C, T-717→A and A-770→G;

T-134→C, A-305→G, A-566→G and T-583→G;

T-583→G;

T-583→A;

T-443→A;

and

T-181→C.

18. (currently amended) The recombinant nucleic acid molecule of claim 16 as contained in a vector ~~such as a plasmid, cosmid, bacteriophage or yeast artificial chromosome.~~

19. (original) A microorganism comprising the nucleic acid molecule of claim 16.

20. (currently amended) The microorganism according to claim 19, wherein the microorganism is selected from the group consisting of: bacteria, yeast, mammalian ~~[[or]]~~ and insect cells.

21. (currently amended) The microorganism according to claim 20, wherein the microorganism is E. coli ~~E. coli~~.

22-26. (cancelled)

27. (currently amended) A method for killing bacteria comprising contacting said bacteria with antibodies to an immunogenic molecule comprising ~~[[the]]~~a modified pneumolysin comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity~~according to claim 1~~ in the presence of complement.

28. (original) The method according to claim 27, wherein the immunogenic molecule is a polysaccharide-polypeptide conjugate wherein the polysaccharide is a bacterial capsular polysaccharide.

29. (currently amended) A method for immunization of mammals comprising administering ~~[[the]]~~a vaccine of comprising the modified pneumolysin polypeptide comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity~~of claim 24~~ and a pharmaceutically acceptable carrier to said mammals.

30. (currently amended) A method for obtaining modified pneumolysin polypeptides, wherein said modified pneumolysin polypeptides have ~~having~~ reduced hemolytic activity and ~~[[being]]~~are suitable for eliciting an immunogenetic response which is cross-reactive with wild-type pneumolysin comprising the steps of:

- (a) ~~randomly~~ mutating a nucleic acid molecule encoding ~~[[for]]~~ wild-type pneumolysin to produce mutated nucleic acid molecules encoding modified pneumolysin polypeptides, wherein the modified pneumolysin polypeptides comprise one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257 and expressing the mutated nucleic acid molecules in host cells;
- (b) assaying the modified polypeptide expressed by the host cells for hemolytic activity; and

- (c) identifying the modified pneumolysin polypeptides having substantially similar molecular weight as native wild-type pneumolysin and which are refoldable.

31. (new) The recombinant nucleic acid molecule of claim 16, wherein the vector is selected from the group consisting of: a plasmid, cosmid, bacteriophage and yeast artificial chromosome.